AN ANALYSIS OF THE ACTION OF LITHIUM ON SEA URCHIN DEVELOPMENT

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The fact that lithium added to sea water profoundly modifies the sea urchin development was discovered by Herbst (1892, 1893, 1895). The most important change involved is the enlargement of the entomesoderm at the expense of the ectoderm. It is important to stress that this shift in the relative amounts of ectoderm and entoderm is not occasioned by any destruction or throwing off of ectoderm material during the early development but by some kind of a change in the determination of the egg. This would mean that the potentialities for producing ecto- and entoderm are so affected by lithium as to favor those which are concerned in the production of entoderm. While Herbst (1893) and Spek (1918) in their interpretation of the "lithium" development directed their main attention towards the vegetative part, MacArthur (1924) and Runnström (1928, 1929, 1933) have emphasized the effect of lithium on the animal part of the larva. All our experimental evidence indicates that the susceptibility to the action of lithium is highest at the animal pole and decreases gradually.

The writer (1929, 1933) has developed the conception that in the sea urchin egg, there exist two opposite gradient systems, one preponderating at the animal, the other at the vegetative pole, as illustrated diagrammatically in Fig. 1. According to this hypothesis, the determination along the egg axis would depend upon the balancing effect between the animal and the vegetative gradient system. The lithium type of development would therefore be due to a shift in the normal balance, as illustrated by Diagram *B* in the figure. The animal gradient system weakens and the vegetative gradient system gains the ascendancy. The ring of skeleton-forming mesenchyme cells appears further towards the animal pole and the boundary between ectoderm and endoderm moves closer to the animal pole of the egg. In extreme cases this condition may be carried to the extent that the entire embryo is transformed into entomesoderm.

Under other conditions the opposite effect is known to occur. For example, by exposing the eggs to certain agents, the vegetative gradient system weakens in comparison to the animal gradient system, cf. Dia-

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gram *C* in the figure. As a consequence of the resulting unbalance, the animal region becomes increased (Herbst, 1904; Lindahl, 1933).

The theory briefly given here is substantiated also by numerous isolation and transplantation experiments carried out by Hörstadius (1935). Only one of these experiments may be mentioned. In the 64-cell stage Hörstadius isolated that one of the two cell rings, originating by the division of the macromeres, which lies at the more vegetative end. This cellular material in normal development gives rise to entoderm only. The isolated ring developed into a larva with both entoderm and ectoderm. Evidently by a regulative process, an almost normal balance had become established in the isolated cell ring. By implantation into this cell ring of one, two, or four macromeres (the most vegetative material), this balance was disturbed and larvæ developed which most strikingly resembled lithium-treated larvæ. Hence an increase in amount of micromere material produced the same effect as an increase in lithium concentration.

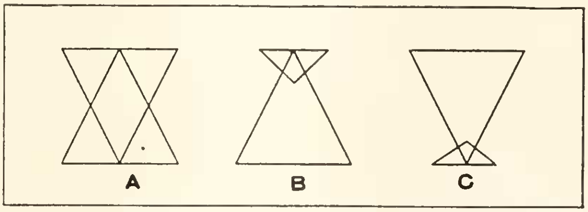

Fig. 1. Diagrammatic representation of the gradient systems in the sea-urchin egg.

The mode of action of lithium has been assumed by Spek (1918) to be a precipitating and swelling effect on the surface of the vegetative cells. This, according to Spek, would account for the "exogastrulation" which is one of the most characteristic effects of treatment with stronger concentrations of lithium. Runnström believes, on the other hand, that such a surface effect is secondary to a more important internal effect and he has experimental evidence to show that lithium penetrates the cells. With dark ground illumination the internal structure of the cells of the "lithium" larva has a coarsened appearance. He assumes that some action of the lithium which is related to this structural change has an effect on the determination and differentiation of the animal pole greater than on the vegetative pole.

Runnström (1933) found that lithium, in concentrations which in themselves are too low to affect development, can be made very active if the eggs, during exposure, are kept in an atmosphere of carbon monoxide containing 5 per cent oxygen. This effect of carbon monoxide is abolished in strong light. The same, as is well known, holds true from Warburg's work (1932) on the effect of carbon monoxide on respiration. This evidence, that the effects of lithium and carbon monoxide are additive, suggests that these two agencies have a similar action on the developing egg. In support of this is the finding of Lindahl (1933, 1934) that lithium has an inhibiting action on the respiration of the egg. Warburg (1915) has shown that a steady increase of respiration takes place during the early development of the sea urchin egg. It is only this increasing fraction of the respiration which is affected by lithium according to Lindahl. This fraction has a special character and may be identical with the oxidation of breakdown products of certain carbohydrates (Lindahl, 1933, 1934, 1935).

During my sojourn at the Marine Biological Laboratory at Woods Hole during the summer of 1934, I had the opportunity of securing the assistance of Professor Robert Chambers in a microdissection study on the physical properties of the eggs of the sand dollar, *Echinarachnius parma,* treated with lithium.

The eggs of this species proved to be extremely sensitive to the action of lithium. A solution of 100 cc. sea water and 5 cc. of 2.6 per cent LiCl killed them in the early stages of segmentation, while the eggs of *Arbacia punctulata* (similar to the European *Paracentrotus lividus*) segment and develop in this solution for more than twenty hours.

It was found that the sand dollar eggs will develop in a weaker solution, for example: eggs immediately after fertilization were placed in 2 cc. of 2.6 per cent LiCl to 100 cc. sea water and, after a sojourn of 20 hours, were transferred to normal sea water. The larvæ exhibited the typical "lithium" effect, namely, a retarded development, a shift of the position of the mesenchyme cell ring and an enlargement of the entodermal region followed by exogastrulation and differentiation of ectoderm and skeleton.

It was found that the physical state of the cells of the larvæ which had been exposed to lithium in sea water was demonstrably different from that of controls developed in sea water alone. The blastulæ were pierced and held in place in the hanging drop (in the microdissecting chamber) by means of one micro-needle and the epithelial wall was torn by means of another needle. In this way strands of the epithelial cells could be stretched. The cells of the controls, after being torn apart and released, quickly became spherical. On the contrary, the cells from the "lithium" blastulæ remained distorted and spindle-shaped. The difference was striking and left no doubt that a profound change in the state of the cytoplasm had taken place as a consequence of the action of the lithium.

It was also found that the hyaline plasma layer which invests the larva is softened and rendered far more pliable when treated with lithium than the layer investing the untreated larva. This fact nullifies any assumption that an increased resistance to the swelling of the blastocœl and the enlargement of the blastula might have been due to a stiffened hyaline plasma layer.

A further observation on control larvæ may be mentioned in this connection. It is easy to observe, in the late blastula or the early gastrula stage, that the hyaline plasma layer presents many folds on the surface of the most vegetative part of the larva. A microneedle could be inserted into this part of the hyaline layer. When the layer was stretched the folds disappeared and a cone-like process was formed. Evidently the folds are formed when the form of the underlying cells changes preparatory to the invagination of cells during gastrulation. The later fate of these folded parts of the hyaline layer is difficult to follow but it is probable that an unfolding takes place when the entoderm cells subsequently enlarge their surface. No studies were made on the presence of folds in the hyaline layer in lithium-treated larvæ, but the folding of the hyaline layer occasionally was observed also in "lithium" larvæ. This speaks also against a stiffening of the hyaline plasma layer under the action of lithium.

The hyaline plasma layer can still be demonstrated even in the normal pluteus. A striking method is to leave the larva in a dish infested with certain Protozoa. These penetrate the dead larva and consume the cellular portion, leaving intact only the skeleton and an investing, fairly soft membrane.

During the development of the larva a considerable growth of the hyaline layer must also take place. This growth must be due to a secreting activity of the cells which normally are kept together chiefly by the hyaline layer. Herbst (1900) has discussed the factors involved in the coherence of the epithelial cells of the sea urchin larva. He considered the action of the hyaline layer and also a second factor, which according to Herbst is more important, and which keeps the cells together even when the hyaline layer has been removed. It seems probable, however, that this second factor is more pronounced only in larvæ which have been submitted previously to a treatment with calcium-free sea water.

During the early differentiation of the sea urchin larva the change in shape of the cells plays an important rôle. Already in the blastula stage a flattening of the cells has begun to take place. In the pluteus most of the aboral part of the ectoderm is formed by a flat epithelium. The indications are that the hyaline plasma layer plays only a subordinate rôle in changing the shape of the cells during development. A retardation in the flattening of the blastula cells is very characteristic for the lithium type of development. The observations made in the course of this work do not favor the idea that the retardation is due to the changed qualities of the hyaline layer. Probably retardation is caused by a " lithium " effect on the internal material of the cells.

The pronounced reaction of *Echinarachnius* eggs to LiCl in comparison with their reaction to chlorides of sodium and potassium are shown in the following experiment. Eggs, recently fertilized, were immersed in solutions of the three chlorides in concentrations approximately isotonic with sea water. In a KCl solution the eggs survived for several hours and underwent cleavage. In NaCl the survival was more limited, for, in the course of an hour, a certain percentage of the eggs underwent cytolysis and the rest at varying times somewhat later. In LiCl all the eggs were destroyed within 10–15 minutes. The surface of the egg burst and a part of the contents flowed into the space under the fertilization membrane while the remainder of the egg shrank somewhat into a clear cytolyzed mass in marked difference to the dark cytolized eggs in NaCl.

The experiments reported in the preceding pages tend to show that lithium has a direct influence on the structure of the cytoplasm. But the possibility is not excluded that the action of lithium, in the range of concentrations producing typical " lithium development," is more indirect. This is rendered probable by the following observations. Eggs of *Echinarachnius* were transferred very soon after fertilization into a solution of 100 cc. sea water containing 5 cc. of 2.6 per cent LiCl and into a similar solution containing 0.5 cc. of 0.1 per cent pyocyanine. In the lithium-sea water the eggs were killed in the two-celled stage but in the lithium-sea water plus pyocyanine a development to the blastula stage took place. In the control the development was normal. This experiment was repeated and varied as to the lithium concentration. It was always found that the addition of pyocyanine caused a marked improvement on the development in lithium-sea water. In a few experiments it was also found that the addition of methylene blue somewhat improves the development in lithium-sea water. From experiments carried out by Friedheim (1931), it is well known that pyocyanine has a promoting action on the respiration of several kinds of cells. Runnström (1935) has shown that this is true also for the fertilized sea-urchin egg. It can thus safely be inferred that pyocyanine acts by inducing oxidation processes which are able to replace, to a certain degree, those suppressed by lithium.

Runnström (1928) found that the addition of KCl to the lithium-sea water in somewhat more than equimolecular concentration to that of the lithium removes the modifying influence of the lithium on morphogenesis. Lindahl (1933) showed that this addition of KCl also removes the inhibitory action of lithium on respiration. From unpublished experiments of mine on *Arbacia* eggs I have found that potassium is more efficient than pyocyanine on the restitution process.

From the facts reported and discussed above it follows that one has not only to consider a general effect of lithium on the physical state of structure but also a specific effect on the metabolism of the sea urchin egg and embryo. This specific effect may possibly be the primary one. These aspects will be more fully discussed in a paper by Lindahl (1935).

Summary

The eggs of *Echinarachnius parma* are very sensitive to the action of lithium added to sea water.

A concentration of lithium was used which produces the typical "lithium development." The epithelial cells of normal and of lithium-treated blastulæ were stretched by microneedles and released. In normal larvæ the cells round up after release; in the lithium-treated larvæ they remain deformed. The hyaline plasma layer does not stiffen in the lithium-sea water and is not necessary for the flattening of the cells which takes place during the development. The presence of folds of the hyaline plasma layer at the vegetative pole in the late blastula and the early gastrula stage is described.

Pyocyanine, added to the lithium-sea water, counteracts the effect of the lithium and improves development. This and other facts indicate that lithium does not only exert an influence on the structure of the protoplasm but also on the respiration.

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